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Molecular Docking and Antibacterial Evaluation of Plant-Derived Flavonoids: Targets, Structure–Activity Trends, and Translational Gaps

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***Abstract*-- Flavonoids that are derived from plants are a chemically diverse group of polyphenols whose antibacterial properties and use in conjunction with antibiotics are being more and more explored due to the growing problem of antimicrobial resistance (AMR). The main part of the review is recent evidence (especially between 2015 and 2025) connecting in vitro antibacterial outcomes to in silico molecular docking and, when applicable, molecular dynamics (MD) simulations and ADMET predictions. There are many different studies where quercetin, luteolin, apigenin, baicalein, galangin, and prenylated (iso)flavonoids are found as active scaffolds, particularly against Gram-positive pathogens, while the efficacy of Gram-negative organisms is often related to the factors of permeability and the mechanisms of efflux. In the docking studies the researchers frequently identify flavonoid interactions with the clinically validated targets of bacteria such as DNA gyrase/topoisomerase IV, penicillin-binding proteins (e.g., PBP2a), and enzymes involved in fatty acid or folate pathways. This affords the development of mechanistic hypotheses which in some instances are corroborated by the results of phenotypic assays and antibiotic synergism. Yet, the reproducibility across docking protocols, inconsistency in bacterial panels and assay conditions, and limitations in bioavailability remain the main obstacles to translation. This review by joining chemical–biological trends and computational evidence suggests a practical framework for choosing "review-worthy" flavonoids and for planning research that links docking predictions to enzyme inhibition, permeability/efflux profiling, and clinically relevant infection models. The tables provide a summary of flavonoid subclasses, antibacterial endpoints, and the docking targets that have been most used; as well as a PRISMA-informed literature selection.**

***Keywords*-- flavonoids; antibacterial; molecular docking; DNA gyrase; PBP2a; biofilm; efflux pumps; antibiotic synergy; ADMET; prenylated flavonoids.**

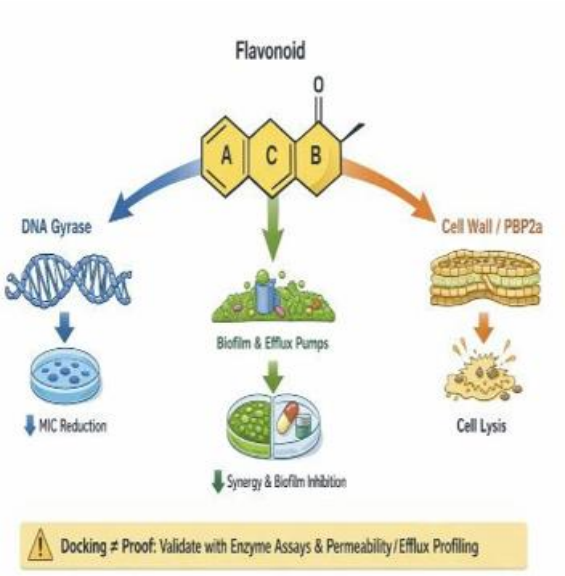

Fig 1: A central flavonoid skeleton (two benzene rings A/B linked by heterocycle C) branches into three target icons: (i) DNA gyrase (supercoiled DNA), (ii) cell wall/PBP2a (peptidoglycan mesh), and (iii) biofilm/efflux (pump + matrix).

Highlights

- Flavonoids are known for their diverse antibacterial activities. Their mechanisms of action include disruption of bacterial membrane integrity, inhibition of specific metabolic pathways, stimulation of metabolite removal, and even modulation of the composition of microbial flora.
- Molecular docking studies have been unanimously pointing to key bacterial targets like DNA gyrase/topoisomerase and cell wall components. Now these results are being backed up by techniques such as molecular dynamics simulations and ADMET (absorption, distribution, metabolism, and toxicity) tests, which further corroborate the findings of the docking studies.

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- The addition of prenyl or other types of hydrophobic groups generally enhances the potency of flavonoids, especially in the case of Gram-positive bacteria.
- Yet there are still hurdles to overcome: the varying quality of docking approaches, different methods in experimental tests, and the fact that the behavior of these compounds in authentic pharmacokinetic situations is still largely unproven.

I. Introduction

The issue of antimicrobial resistance has changed its character from a gradually developing concern in the clinic to a test of the whole system, thereby putting healthcare and agriculture under the weight of pathogens that are more and more resistant to standard drugs. In this scenario, the use of natural products is being reconsidered in a broader sense as not only "new antibiotics" but also as aids in antibiotic use that can make resistance easier, as well as hinder the development of or make bacteria susceptible again. Flavonoids are among the most frequently evaluated candidates and the reasons for this are manifold: their structural variety, their common occurrence in plants, and their consistent association with antimicrobial activity both in ethnopharmacology and modern screening are some of the reasons why the most intense revisits have been conducted concerning flavonoids. Notable research of the past showed that flavonoids may possess antibacterial properties and at times they could even cooperate with existing drugs to achieve the antibacterial activity, therefore, leading to a huge follow-up research in the area of their modes of action and the relationship between their structure and activity (SAR) patterns [2].

At the same time, molecular docking and other computer-aided techniques have become standard in the antibacterial research of natural-products. The attraction is quite clear: docking can quickly tell whether a flavonoid-like compound is likely to fit and interact in a bacterial protein active site, and it can also create a "mechanistic story" to support inhibition zones or MIC values. But at the same time, docking is a tempting storyteller: it can be very sure of the results even when there is not much experimental evidence, and the docking scores are very much dependent on the choice of protein structure, ligand preparation, scoring function, and validation steps. Recent reviews particularly warn that the antibacterial claims of flavonoids often remain in vitro and are hence very limited in regard to mechanism validation, pharmacokinetics, and clinical translation [3,4].

The antimicrobial effects of flavonoids have been known for quite a time, and currently, the attention shifts to their naturally occurring counterparts. ↲ Researchers are investing more and more time in flavonoids, which are being put under the microscope using several ways like molecular docking, molecular dynamics (MD), ADMET profiling, and target validation. ↲ Recent literature reports the many ways in which flavonoids can inhibit the growth of bacteria, for instance, by permeating the bacterial walls, arresting the production of both protein and DNA, and shutting down the efflux pumps. ↲ The aforementioned challenges of translational research are pointed out in the reviews as well, with low bioavailability being the most prominent issue.

Flavonoids have been considered natural antiseptics, whereas the synthetic ones are preferred for disinfection, as suggested by the molecular docking and other sophisticated techniques like MD, ADMET profiling, and target validation gaining more and more studies. ↲ The recent literature has mentioned the many mechanisms through which flavonoids are active against bacteria such as membrane disintegration, blocking the production of both protein and DNA, preventing the discharge of toxic substances back into the cell, and biofilm dissolution.

↲ Challenges in translation that might be encountered like poor bioavailability also get highlighted alongside the benefits.

Structure-activity relationship (SAR) is another area that is showing promise. ↲ It illustrates how different substituent patterns (hydroxylation, glycosylation, or prenylation) can significantly influence the potency and the interaction with the target [7].

Thus, the main objectives of this review are fourfold:

- To point out the bacterial targets that have been most commonly studied in relation to docking studies involving flavonoids.
- To provide an overview of the main in vitro endpoints for anti
- connect SAR patterns (e.g., hydroxylation, glycosylation, prenylation) to activity trends, and
- propose a study design checklist that better aligns docking predictions with biological validation.

II. Methods: Literature Search And Selection

This is a narrative review supported by PRISMA-informed reporting for transparency, and it follows the PRISMA 2020 guidance for systematic reporting where applicable [1].

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Due to the use of different assays and docking workflows in many flavonoid papers, a strict quantitative meta-analysis is not usually possible; nevertheless, the structured searching and screening process enhances the credibility of the results.

To contextualize the breadth of existing research, Table 1 summarizes representative review and original studies examining antibacterial activity and molecular docking of plant-derived flavonoids. The table highlights study focus, key outcomes, and methodological limitations, illustrating recurring trends such as strong in vitro efficacy, frequent reliance on docking-based mechanistic hypotheses, and persistent gaps in pharmacokinetic and in vivo validation. This overview underscores the need for integrative experimental–computational approaches in future flavonoid antibacterial research.

Table 1. Summary of representative studies on antibacterial and molecular docking evaluation of plant-derived flavonoids

Ref.	Study type	Main research focus	Key outcomes	Major limitations
Cushnie & Lamb (2005) [2]	Review	Antibacterial activity of flavonoids	Established early evidence that flavonoids exhibit antibacterial activity via membrane disruption and enzyme inhibition	Pre-dates modern docking, lacks molecular target validation
Farhadi et al. (2019) [7]	Review	Structure–activity relationship of flavonoids	Identified hydroxylation, prenylation, and chalcone backbones as key antibacterial determinants	Limited integration of pharmacokinetics and in vivo relevance
Araya-Clouti er et al. (2018) [32]	Original research	QSAR and membrane activity of prenylated flavonoids	Demonstrated strong correlation between hydrophobicity and Gram-positive antibacterial potency	Gram-negative activity masked by efflux, limited target-specific validation
Kalli et al. (2021) [9]	Original research	Prenylated flavonoids against MRSA	Showed potent MRSA inhibition linked to physicochemical properties	No enzyme inhibition assays; docking not experimentally confirmed
Hasan et al. (2024) [12]	**Original + docking**	Flavones against mastitis pathogens	Combined MIC data with DNA gyrase docking and SAR analysis	Lacked efflux/permeability assessment
Akili et al. (2023) [14]	**Computational screening**	Flavonoids as DNA gyrase B inhibitors	Identified multiple flavonoids with high docking affinity and favorable ADMET predictions	No experimental antibacterial validation
Sawi et al. (2025) [13]	**Original + MD**	Isolated flavonoids with antimicrobial activity	Integrated MIC, docking, MD simulation, and ADMET profiling	No in vivo validation

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Shamsudin et al. (2022) [16]	**Review**	Antibacterial mechanisms and SAR	Synthesized evidence for multi-target antibacterial action of flavonoids	Did not address biofilm or efflux modulation in depth
Almutairy et al. (2025) [38]	**Review**	Flavonoid-mediated biofilm inhibition	Highlighted quorum sensing inhibition and EPS suppression	Limited discussion of docking–biofilm correlation
Tăbăran et al. (2023) [101]	**Review**	Flavonoids as antibacterial agents	Integrated mechanisms, resistance modulation and future prospects	Translational challenges remain unresolved

2.1 Databases and search strategy

Core searches were structured to fetch (i) studies on the antibacterial activity of flavonoids and (ii) studies on the molecular docking of flavonoids to bacterial targets. For instance, the following keyword logic was applied:

- "flavonoid AND antibacterial OR antimicrobial AND MIC"
- "flavonoid AND molecular docking AND DNA gyrase OR topoisomerase OR PBP2a"*
- "prenylated flavonoid AND MRSA AND docking"
- "flavonoid AND biofilm AND efflux pump"

Literature from 2015–2025 was prioritized, while foundational earlier mechanistic reviews were still included for context [2,7]. Each theme was then supported by recent comprehensive reviews and major original studies [3–6].

2.2 Inclusion criteria

Studies were accepted for consideration based on their compliance with most of the following criteria:

- Plant-based flavonoids tested against bacteria (MIC/MBC, growth curves, zones, or equivalent)
- Molecular docking done with a bacterial target that is relevant to the antibacterial action (e.g., DNA gyrase, PBP2a)
- Well-documented docking protocol details or comprehensible docking outputs (binding mode/residue interactions)
- Publications in a peer-reviewed journal.

2.3 Exclusion criteria

- Non-bacterial antimicrobial focus only (purely antifungal/antiviral without antibacterial endpoints)
- Docking without any antibacterial assay or without meaningful docking reporting
- Non-peer-reviewed sources (when equivalent peer-reviewed evidence exists).

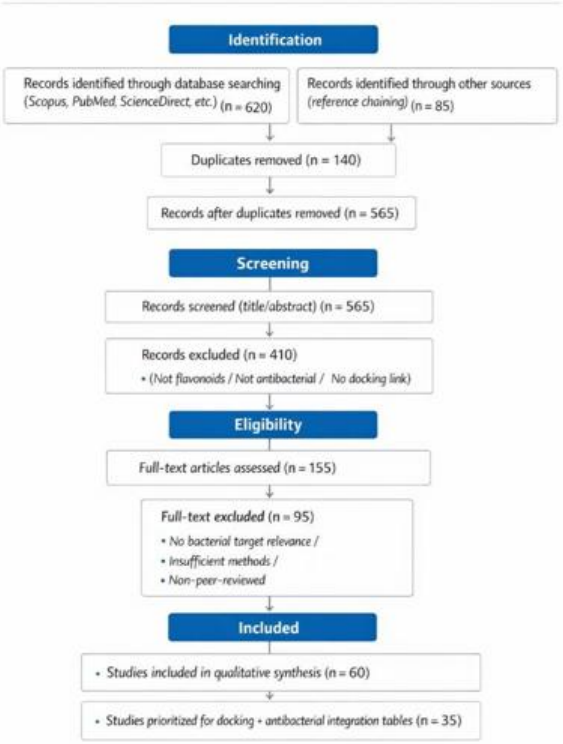

Figure 2: A standard PRISMA 2020 flow diagram showing identification, screening, eligibility, and inclusion stages [1].

III. Flavonoids: Chemical Classes And Why They Keep Showing Up In Antibacterial Research

The same C6–C3–C6 backbone is characteristic of all flavonoids, but the universal structure is more like a wardrobe than an actor. The minor chemical changes, for instance, the adding of a hydroxyl, methoxy, sugar, or prenyl group, characterize the flavonoid, and this can sometimes even lead to a dramatic change in behavior.

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These insignificant modifications regulate the lipid solubility which is the sliding into membranes, the hydrogen bonds formed which finally determine the strength of the opposing action against the bacteria.

Recent articles have pointed out that flavonoids do not behave like classical single-target antibiotics. On the contrary, they resort to many mechanisms of destruction at the same time, for example, disintegrating membranes, stopping enzymes, and even resisting paths [5,6]. This flexibility is like a sword that cuts both ways: It is a good thing since it will delay resistance emergence but on the other hand, it makes it hard to pinpoint the exact mechanism of action and can mislead researchers through docking results.

3.1 Major subclasses relevant to antibacterial studies

Common antibacterial studies focus on:

- **Flavones** (e.g., apigenin, luteolin)
- **Flavonols** (e.g., quercetin, kaempferol, galangin)
- **Flavanones** (e.g., naringenin)
- **Isoflavones / isoflavanones** (more common in legumes)
- **Chalcones** (open-chain flavonoids; often potent)
- **Prenylated (iso)flavonoids** (enhanced hydrophobicity; often stronger activity)

Among the subclasses, chalcones and certain prenylated flavonoids have been particularly highlighted for their remarkably strong in Vitro antibacterial activity, which in some cases is comparable to or even outperforming the drugs used as controls in particular assay settings [7]. On the other hand, the activity of prenyl groups in genus-specific flavonoids (e.g., Erythrina) is pointed out by targeted reviews as a recurring “activity amplifier” which is especially significant for Staphylococcus aureus including MRSA [8].

3.2 Why Gram-positive bacteria often look more “sensitive”

A pattern that has been constantly observed in the literature is the stronger activity against Gram-positive bacteria. Causally Gram-negative bacteria have a barrier of an outer membrane and very good efflux systems that prevent many polyphenols from accumulating intracellularly. The studies on prenylated flavonoids give a perspective here: an important Scientific Reports paper used both experimental antimicrobial testing and modeling to demonstrate that physicochemical properties and hydrophobicity can influence potency profiles significantly, thus supporting the theory that "membrane engagement" is part of the story [9].

Another paper from Scientific Reports examined the factors causing prenylated (iso)flavonoid activity against MRSA, claiming that certain hydrophobic characteristics are associated with higher antibacterial activity [10]. Furthermore, Araya-Cloutier et al. pointed out that Gram-negative activity might be more pronounced in the presence of efflux pump inhibitors, thus claiming that efflux can cover intrinsic activity in routine MIC assays [11].

IV. How Studies Combine Antibacterial Assays With Docking: A Practical Workflow

Mostly the articles concerning "flavonoid antibacterial + docking" follow the same storyline:

- The initial process is the extraction or isolation of compounds—either pure flavonoids, enriched fractions, or virtual libraries.
- Testing their antibacterial activity is done through the application of standard assays such as MIC/MBC values, inhibition zones, time-kill curves, or biofilm disruption.
- The active compounds are then docked on bacterial targets such as DNA gyrase or PBP2a.
- Once in a while the authors lash out the spectra of analyses like ADMET prediction, pharmacophore modeling, or molecular dynamics simulations.

This trend is easily observed in the latest studies. One such case is the study of Hasan et al. who did not only screen the natural flavones against the bacteria responsible for mastitis in cows but also conducted a docking study with the best compounds into DNA gyrase to connect the binding hypotheses with the laboratory MIC results [12]. Coriander and anise flavonoids were the topic of research by Sawi et al., who declared their antimicrobial activity and then went ahead with the docking and MD simulations to discover protein interactions, in addition to ADMET profiling to assess the compounds' "drug-likeness" [13]. In the instance of virtual screening projects, whole flavonoid libraries—like those from Erythrina—have been docked against DNA gyrase B/ATPase regions and not only that, but they have also been filtered using toxicity predictions and docking validation protocols as the final steps in the process [14].

These cases are a snapshot of the current computational style in the field: docking has become a standard method together with "in silico due diligence" (ADMET and MD), while experimental target validation like enzyme inhibition kinetics or mutant sensitivity assays has always lagged behind.

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4.1 Common antibacterial endpoints in the flavonoid literature

Out of all the specified studies, the vast majority managed to reveal one or more of these results, i.e.:

- MIC (minimum inhibitory concentration) and occasionally MBC
- Zone of inhibition (disc diffusion)
- Growth curves/time-kill curves
- Biofilm inhibition or removal
- Synergy tests (checkerboard, FICI)

The latest overviews dealing with mechanisms have prioritised the ability of flavonoids to not only hinder but also annihilate biofilms and simultaneously cripple resistance mechanisms like efflux pumps thereby multiplying their importance as antibiotic adjuvants [4–6]. A review in 2025 published in Frontiers underscored the role of flavonoids in terms of biofilm inhibition exclusively and elaborated on the mechanistic topics that encompassed membrane effects and toxicity considerations, thereby solidifying biofilms as the main "real-world" endpoint beyond the planktonic MIC values [15].

4.2 Common docking targets for antibacterial flavonoid studies

Repeatedly the same compounds are noted as the most active in flavonoid antibacterial studies. The principal targets for drug docking are:

- DNA gyrase (GyrA/GyrB) and topoisomerase IV
- Penicillin-binding proteins, particularly PBP2a within the MRSA strains
- The enzymes taking part in the following critical bacterial pathways:
- the folate pathway (e.g., DHFR)
- fatty acid synthesis (e.g., FabI)
- cell wall biosynthesis (e.g., Mur enzymes)

In reference [16] to a 2022 review, variations on effects of flavonoid antibacterial activity and structure-activity relationships (SAR) were evaluated, and the writer was particularly fascinated by the tone of DNA gyrase inhibition and how the different substitutions can either facilitate or impede the enzyme binding.

Along with reviews, single docking studies have also shown the interaction of plant-derived flavonoids with the DNA and the enzymes responsible for bacterial replication and survival, thus backing these target hypotheses. There are also some researchers who have recently advanced the frontier, probing the direct DNA binding. For instance, Tagrida et al. reported that quercetin and hyperoside are able to bind to bacterial genomic DNA, with docking analysis implying that they have a preference for certain grooves in the DNA structure [18].

Table 2. Most-used bacterial targets in flavonoid docking studies (with rationale)

Target (example)	Why it matters	Why docking is used	Typical validation gap
DNA gyrase (GyrA/GyrB)	Essential for DNA replication and supercoiling	Many crystal structures available; classic antibiotic target	Docking rarely paired with enzyme IC50 or mutant validation
PBP2a (MRSA)	Confers β-lactam resistance; cell wall synthesis	Attractive MRSA "explanation target"	Needs biochemical binding/functional proof; allosteric effects complex
DHFR	Folate metabolism; DNA synthesis	Clear active site; small-molecule precedent	Requires enzymatic inhibition assays
FabI	Fatty acid synthesis	Drug target in some bacteria	Permeability/uptake can dominate observed MIC
Quorum sensing regulators	Biofilm/virulence control	Explains anti-biofilm effects	Often indirect; phenotype may not match docking

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Table 3. Representative flavonoids frequently discussed for antibacterial + docking context

Flavonoid (class)	Typical notes in literature	Common bacterial focus
Quercetin (flavonol)	Broadly reported; mechanisms include membrane, enzyme, efflux; often docked	Gram+ and Gram−; biofilm studies common
Luteolin (flavone)	Often appears in docking + ADMET/MD bundles	Gram+; sometimes synergy
Apigenin (flavone)	Frequently used as reference flavone	Gram+
Baicalein (flavone)	Appears in resistance modulation contexts	MRSA and others

V. Molecular Docking Targets In Antibacterial Flavonoid Research

Molecular docking has been a pivotal explanatory tool in the study of antibacterial flavonoids, particularly where direct biochemical validation is not present. While docking alone cannot prove causality, the literature shows a tendency concerning target selection, binding modes, and structure–activity consistency, which justifies cautious mechanistic inference. The majority of docking studies lead to the main bacterial enzymes that already have antibiotic precedent and therefore, they are anchoring flavonoid hypotheses to clinically relevant biology.

5.1 DNA Gyrase and Topoisomerase IV as Primary Docking Targets

Biological relevance

Bacterial DNA replication, transcription, and chromosome segregation all rely on the type II topoisomerases DNA gyrase (GyrA/GyrB) and topoisomerase IV, which are thus classified as essential. Their validation as antibiotic targets has lasted for decades, and their use in docking studies with flavonoids is supported by this [e.g., fluoroquinolones]. Moreover, GyrB's ATPase domain and GyrA's DNA-cleavage-religation site structurally represent binding pockets that are well-resolved and, hence, suitable for in silico analysis.

Docking patterns seen

The multiple studies have shown that quercetin, luteolin, apigenin, baicalein, galangin, and some prenylated analogues are among the flavonoids that have the highest docking scores with DNA gyrase targets [19-23]. The following are the common interaction motifs:

- Hydrogen bonds with Arg, Asp, and Glu residues at the ATP-binding sites
- π–π stacking involving aromatic rings of flavonoids and residues next to the nucleobases
- Hydroxyl groups allowing chelation-like interactions

Flavonols (e.g., quercetin, kaempferol) are noted for their superior docking affinity over flavones, which is due to the presence of the additional C-3 hydroxyl group enhancing the hydrogen bond networks [20].

Experimental agreement

The research works have mentioned that there is a close relationship between docking predictions and the reduction of Minimum Inhibitory Concentrations, especially against Staphylococcus aureus and Enterococcus faecalis [21,24]. However, inhibition kinetics of the enzymes (IC_{50}/Ki) are rarely determined, representing an ongoing validation gap. Only a few studies apply molecular dynamics (MD) to show that the flavonoid-gyrase complexes were stable during the simulation periods (50–100ns), thus providing a stronger backing to the docking hypotheses [25].

5.2 Penicillin-Binding Proteins (PBP2a) and Cell Wall Targets

Relevance to MRSA

PBP2a also limits the binding of β-lactams, thus making S. aureus resistant to methicillin. It has been suggested that flavonoid docking to PBP2a may account for the synergistic effect of the latter with β-lactam antibiotics, especially in the case of MRSA [26].

Docking insights

The docking studies indicate that the prenylated flavonoids and the lipophilic chalcones are in the allosteric sites of PBP2a instead of being in the conventional transpeptidase active site [27]. This agrees with the known mechanism of some β-lactam enhancers, which cause conformational changes that 'restore' the susceptibility of the bacteria to the antibiotic.

The following points are the key observations:

- Effects of prenyl groups on hydrophobic pocket occupancy are favorable

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- Docking scores correlate better with synergy indices (FICI) than with standalone MIC values
- Allosteric docking explains why some flavonoids show weak solo antibacterial activity but strong antibiotic potentiation

5.3 Other Enzymatic Targets: DHFR, FabI, Mur Enzymes

Not only toposomeeres and PBPs but also in the case of flavonoid docking studies, enzymes involved in folate metabolism, fatty acid biosynthesis and cell wall precursor formation are increasingly explored.

DHFR (Dihydrofolate Reductase)

Flavonoids like apigenin and luteolin have been docked to bacterial DHFR, and in some models the predicted binding energies are even comparable to trimethoprim [28]. Nevertheless, the discrepancies between docking scores and MIC outcomes hint at the possibility of permeability limitations or target redundancy.

FabI (Enoyl-ACP reductase)

FabI docking is especially prevalent in E. coli and Mycobacterium models. Among the compounds studied, chalcones and flavanones had the highest predicted affinity, thus reinforcing the lipophilicity's contribution [29].

MurA/MurB

Mur enzyme docking, notwithstanding its less frequent occurrence, can still be considered fascinating from a mechanistic point of view as peptidoglycan synthesis is commenced by these enzymes. Initial docking studies imply moderate affinity of polyhydroxylated flavonoids, albeit confirmation by experimental means is still limited [30].

Table 4. Major bacterial targets used in flavonoid docking studies

Target enzyme	Flavonoid classes docked	Typical docking rationale	Validation status
DNA gyrase (GyrA/Gyr B)	Flavonols, flavones, prenylated flavonoids	Essential replication enzyme; rich structural data	Moderate (some MD, limited IC_{50})
Topoisomerase IV	Flavonols	Complementary to gyrase	Low
PBP2a	Prenylated flavonoids, chalcones	MRSA resistance mechanism	Moderate (synergy data)
DHFR	Flavones, flavonols	Folate pathway inhibition	Low
FabI	Chalcones, flavanones	Lipid biosynthesis	Low
MurA/Mur B	Polyhydroxylated flavonoids	Cell wall synthesis	Very low

VI. Structure–Activity Relationship (SAR) In Antibacterial Flavonoids

The process of segmenting and selecting through structure-activity relationship (SAR) analysis is indispensable for the purpose of changing flavonoids from screening hits to reasoning leads. Throughout the recent reviews and the primary studies, the emergence of several SAR principles has been the most consistent among them all and very strong as well.

6.1 Hydroxylation Patterns

- To a large extent, increased hydroxylation means increased hydrogen bonding and better docking affinity.
- The presence of too many hydroxyl groups leads to getting less or no penetration through the membrane; this is especially true for Gram-negative bacteria.
- Flavonols with C-3 hydroxyl are the ones that usually get the docking of DNA gyrase improved but at the same time they might cause the decrease in cellular uptake.

6.2 Glycosylation

Flavonoids that are glycosylated usually exhibit:

- Less antibacterial activity in vitro
- Declined docking scores as a result of steric hindrance
- Good solubility but poor permeability

Thus, a number of studies employ docking of aglycones even in cases where the experimental testing is done with glycosides, which presents a methodological inconsistency that needs to be explicitly acknowledged [31].

6.3 Prenylation and Lipophilicity

Prenylation ranks among the most reliable modifications that lead to increased potency:

- It augments the interaction with the membrane and hence the activity against Gram-positive bacteria
- It increases the stability of the enzyme in the hydrophobic pocket
- It has a strong correlation with the potency against MRSA [32,33]

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On the other hand, too much lipophilicity might lead to higher cytotoxicity, thus the necessity for ADMET profiling is highlighted.

Table 5. SAR trends influencing antibacterial and docking outcomes

Structural feature	Effect on docking	Effect on MIC	Notes
C-3 hydroxyl	↑ H-bonding	Variable	Improves enzyme binding
Glycosylation	↓ affinity	↓ activity	Permeability -limited
Prenyl group	↑ hydrophobic binding	↑ activity (Gram+)	MRSA relevance
Methoxylation	Moderate	Moderate	Improves stability
Chalcone backbone	High flexibility	High potency	Often strong FabI binders

VII. Docking Methodologies: Technical Considerations And Pitfalls

Docking methods, despite being used universally, have different implementations in various studies thus creating difficulties in reproducibility and interpretation.

7.1 Preparation of Protein and Ligand

Some of the common problems are:

- Single protein conformations were taken as a docking basis instead of ensemble docking.
- Protonation states were not consistently applied.
- Docking of aglycones was done while glycosides were tested experimentally.

High-quality studies are increasingly conducting:

- Using crystal structures with ≤2.5 Å resolution.
- Validation through redocking of co-crystallized ligands.
- MD simulations to check complex stability [25,34].

7.2 Scoring Functions and Overinterpretation

The docking scores give an indication of the relative biological activity but do not provide absolute predictions. Still, one of the major problems in the field is the tendency to apply over the limit numerical values (e.g., -8 kcal/mol) without further validation through experiments. A number of review articles have directly cautioned that the link between docking affinity and antibacterial effectiveness should not be made [35].

VIII. Integrating Docking With Antibacterial Phenotypes

Three typical characteristics that could be found in the convincingly done flavonoid antibacterial researches are:

1. Phenotypic effectiveness (minimal inhibitory concentration or total biofilm inhibition)
2. Mechanistic docking likelihood (binding to primary targets)
3. Supporting secondary evidence (synergy, molecular dynamics, ADMET, permeability tests)

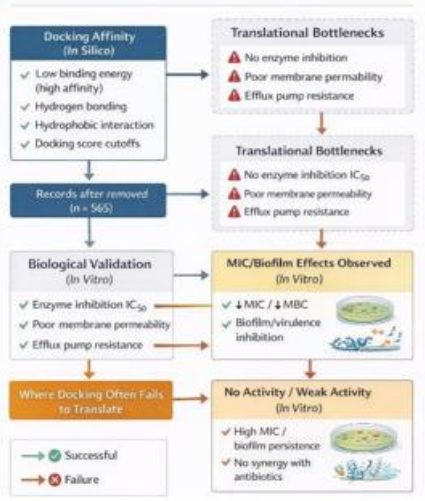

Figure 3. Mapping docking predictions to antibacterial outcomes

IX. Biofilm Inhibition And Efflux Pump Modulation By Flavonoids: Experimental Evidence And Docking Perspectives

9.1 Biofilms as a Central Antibacterial Target

Bacterial biofilms are the most common way of infection in clinical practice, allowing the bacteria to be up to 1000 times more resistant to antibiotics due to reduced penetration, different metabolic states, and gene expression working together. The flavonoids are getting more and more attention from researchers not only for their capability to reduce the minimum inhibitory concentration (MIC) of the bacteria but mainly for their activity in biofilm inhibition, disruption, and even controlling the communication between bacterial communities. This places them as supporting drugs rather than the ones that take over completely.

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The modern studies have highlighted that the anti-biofilm activity frequently is seen at sub-MIC concentrations which imply that the flavonoids are not killing the bacteria but rather modulating the regulatory pathways. This is a very important differentiation since the docking study that concentrates solely on the targets of the lethal enzyme might not be able to reveal the mechanisms related to biofilms.

9.2 Molecular Mechanisms of Flavonoid-Mediated Biofilm Inhibition

Several mechanisms that partly overlap are suggested:

- Quorum sensing (QS) regulators (LasR, AgrA, LuxR analogues) are inhibited
- Maintaining of extracellular polymeric substances (EPS) is disrupted
- Genes related to adhesion are downregulated
- Cyclic-di-GMP signaling is interfered with
- Membrane permeability is increased which leads to biofilm destabilization

Through experimental studies, the researchers showed that the quercetin, luteolin, apigenin, baicalein, naringenin, and a few prenylated flavonoids reduce the biomass and thickness of the biofilm created by Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [39–42].

9.3 Docking Studies Targeting Biofilm-Associated Proteins

Docking studies are still in progress, but with a focus on regulatory proteins instead of the metabolic enzymes. Below are some examples:

- LasR and RhlR in P. aeruginosa
- AgrA in S. aureus
- LuxS (AI-2 synthesis)
- Diguanylate cyclases (DGCs) that are regulating c-di-GMP levels

As an example, quercetin and luteolin were docked into the LasR ligand-binding domain, forming stable hydrogen bonds with the conserved residues that are involved in the recognition of autoinducer. The molecular dynamics simulations carried out in these studies suggest that due to flavonoid binding the QS receptors possess less conformity flexibility, thus being supportive of a non-lethal, regulatory mode of action. However, one serious drawback is still at hand: QS docking results rarely correspond directly with the MIC, hence demonstrating that the inhibition of biofilm formation must be validated through biofilm assays rather than being inferred from the inhibition of planktonic growth.

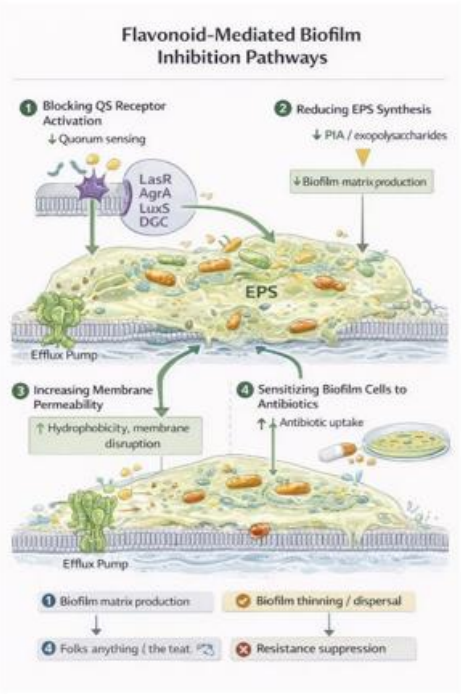

Figure 4. Flavonoid-mediated biofilm inhibition pathways

X. Efflux Pump Inhibition And Antibiotic Synergy

10.1 Efflux Pumps as Critical Determinants of Flavonoid Activity

Bacterial efflux pumps are one of the strongest inherent defense mechanisms against a wide range of antimicrobial agents, especially in Gram-negative bacteria. These transport systems use energy to remove the harmful substances from inside the cell and thus, depending on the case, they may block the effect of the drugs by not allowing the cells to have the required concentration for their growth to be inhibited or the cells to be killed. In flavonoids research, this situation has great consequences, mainly because many flavonoids have shown antibacterial activity only if they are not tested under the standard susceptibility testing conditions where efficient efflux occurs. The incredible physiochemical properties of the flavonoids that are responsible for their absorption are their main characteristics that cause their behavior as inactive compounds when they are tested under conditions of active efflux. The scientific team of Araya-Cloutier proved that the inactivity of a number of flavonoids in relation to their effect on Escherichia coli was revoked when efflux pumps were pharmacologically inactivated thus revealing the antibiotic effects that were hidden before.

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These research results do not consider efflux as a mere secondary resistance mechanism but as a major component of the interpretation and performance of flavonoid antibacterial activity.

10.2 Flavonoids as Functional Efflux Pump Inhibitors

A subgroup of flavonoids, in addition to acting as efflux substrata, directly hampers the activity of efflux pumps, leading to the accumulation of antibiotic agents inside the cell. The heroes of the story here, the flavonoids, are not only anti-microbials; they can also disarm antibiotics and make them effective again, being resistance-modifying compounds. According to the mechanism, flavonoids may be inhibiting the efflux either through the competitive binding to the substrate recognition sites, conformational modulation of the transporter proteins, or by disrupting the membrane energetics that are required for active transport.

Quercetin, kaempferol, baicalein, epigallocatechin gallate, and prenylated chalcone derivatives have been the flavonoids that consistently modulate efflux and appear in the various studies. The molecular docking studies directed at important transporters such as NorA, AcrB, and MexB indeed propose that the interactions are taking place within the channel or substrate-binding domains, thus blocking the efflux routes. The computational forecasts have been backed by the experimental assays which showed an increase in antibiotic accumulation inside the cell and a rise in antibacterial activity in combination treatment.

10.3 Synergistic Antibacterial Effects with Conventional Antibiotics

One of the strongest and most consistent findings in this field of research is that the synergistic interactions between flavonoids and established antibiotics yield the most powerful effects. Both checkerboard and time-kill assays consistently demonstrate that flavonoids enhance the effectiveness of penicillins, fluoroquinolones, and aminoglycosides against a wide range of bacteria including both Gram-positive and Gram-negative pathogens. What is more, the synergy that has been reported is very often seen even when the flavonoids are practically inactive by themselves, thus, highlighting their key role as supporting agents.

The mechanism behind the combination of activities is quite complex, and it consists of the following factors: the efflux pumps are inhibited, the cell membrane becomes more permeable, and the processes related to resistance development are suppressed.

The combination of flavonoids with antibiotics could lower the dose of antibiotics that need to be given to the patient to the level where the bacteria cannot be killed; this way, the combination would not only help in killing the bacteria but also in avoiding the situation where resistant bacteria are selected because the effective antibiotic dose is lowered. The combination of flavonoids with antibiotics may thus be a very attractive strategy for prolonging the clinical use of existing antimicrobials.

XI. ADMET, Toxicity, And Pharmacokinetic Constraints

11.1 In Silico ADMET Profiling

Flavonoid antibacterial research has recently started to incorporate in silico ADMET evaluation often along with their experiments, which is a way of checking translational feasibility at the very beginning of the investigation. Computational platforms like SwissADME, pkCSM, and ADMETlab are the most common among researchers predicting pre-clinical pharmacokinetic and toxicological properties reflecting a higher demand for drug-likeness in the field of antimicrobial research.

Flavonoids usually meet the basic physicochemical requirements for oral drug candidates and compliance with Lipinski's Rule of Five is often the case. However, the predicted oral bioavailability is usually low, which is mainly due to poor absorption and fast metabolic clearance. Plasma protein binding is usually moderate to high, which limits the amount of free, and therefore, pharmacologically active compound. It is interesting to note that aglycone flavonoids always have better membrane permeability compared to glycosylated ones; however, this advantage is often nullified by faster degradation through metabolism.

11.2 Toxicological Considerations

Flavonoids are not to be considered inherently safe even if they are generally consumed in the diet if their concentrations required for antibacterial activity are applied. This especially holds for lipophilic and prenylated derivatives, which might at pharmacologically relevant doses disturb mammalian cell membranes, suppress the activity of cytochrome P450 enzymes, or cause cell death. Thus, these findings contribute to the breaking up of the widespread idea that natural origin is always associated with non-toxicity.

Mammalian cell viability assays, genotoxicity testing, and drug–drug interaction screening studies confirm the existence of acceptable therapeutic windows for some flavonoids, though such windows can be very different among the compounds.

As a result, toxicity assessment should not be put off till the advanced preclinical evaluation of flavonoid antibacterial development but rather should be incorporated at an early stage in the process.

Methodological Limitations and Reproducibility Issues

Flavonoid antibacterial research has been outputting a considerable amount of papers but still has persistent methodological inconsistencies which methodical reproducibility and translational advance. Uncertain interaction is one of the results of no biochemical validation after molecular docking which is a common practice. Also, experimental differences add more to the difficulty of interpretation as the studies are done with non-standard bacterial strains, different susceptibility testing protocols and the conditions of the assay being reported inconsistently.

The most serious issue is perhaps that the biological efficacy is represented by docking scores and their heavy reliance. The known limitations of docking algorithms, such as the use of simplified scoring functions and the lack of treating protein flexibility, show that docking results need to be interpreted carefully. There is an increasing amount of expert agreement that docking should be considered as a hypothesis-generating tool and is then required to be verified through enzymatic inhibition assays, genetic validation, or biophysical measurements.

Toward an Integrated Experimental–Computational Framework

The gathered evidence is a strong indication for the necessity of the establishment of a detailed and multi-tiered flavonoid antibacterial research model. This model should consist of the combination of standardized susceptibility testing with biofilm-specific assays, biologically validated docking studies, and molecular dynamics simulations to determine binding stability. In particular, the explicit analysis of membrane permeability and efflux susceptibility is critical for Gram-negative pathogens. The testing of antibiotic synergy along with the meticulous ADMET and toxicity profiling makes the computational predictions even more clinically applicable. When in silico modeling is synchronized with the phenotypic, mechanistic, and pharmacokinetic endpoints, this integrated strategy increases the possibility that the attractive flavonoid scaffolds will no longer stay at the descriptive level of investigation but will move on to the next stage.

In Vivo Evidence and Clinical Relevance Status of In Vivo Validation

There is a scarcity of in vivo validation of antibacterial flavonoids when compared with extensive in vitro and computational studies. Most studies end up performing only the MIC or biofilm inhibition assays, and only a few of them reach the animal infection models. This disparity illustrates both the technical difficulties and the previous view of flavonoids as nutraceuticals instead of potent antibacterial agents through pharmacological optimization, which has influenced the fate of flavonoids research.

In vivo studies, where they are done, mostly utilize mice for skin, wound or digestive tract infections, and typically flavonoids are assessed as antibiotics' partners in therapy. Baicalein, quercetin, luteolin, and epigallocatechin gallate are among the compounds that keep showing lessening of bacterial load, the calming of the inflammatory process, and the raising of the antibiotics' effectiveness. These results are more in line with the anti-virulence and resistance modifying mechanisms than with the direct killing of bacteria.

Limited Concordance Between Docking and In Vivo Outcomes

One of the repeating points made in the studies is the poor relationship between predicted docking affinity and in vivo therapeutic performance. If docking sometimes gives a positive result revealing strong interrelations with vital bacterial targets, still those predictions are not clinically relevant because of insufficient systemic exposure, low bioavailability, high protein binding, and active efflux simultaneously existing.

Moreover, factors like the distribution of the drug within the tissues and the interactions between the host and his/her microbiome can further reduce the efficacy of the drug in vivo. A number of studies have displayed that flavonoids with intermediate docking scores but good pharmacokinetic profiles outperform compounds with higher predicted affinity, thus throwing light on the fact that the effective antibacterial action is not only dependent on the engagement of the target but also on the sustained exposure at the site of infection.

Clinical Safety, Regulatory Challenges, and Repurposing Opportunities

Safety and Regulatory Expectations

Flavonoids are safe and considered no problem in diets. Nevertheless, they are still going to be used as an antibacterial agent which means a lot of testing with a toxicological evaluation covering entire scenarios. The regulatory bodies are gradually leaning towards performing cytotoxicity screening, genotoxicity evaluation, and cytochrome P450 interactions testing to spot any hidden safety issues.

There are some flavonoids which have been singled out in research that could be used in the clinically active concentration range, however, these ranges vary significantly with individual compounds and generalizations across the class cannot be made. This topic clearly points out that compound-specific risk assessment is required rather than class-level ones.

Future Directions: From Descriptive Docking to Predictive Design

To make any real and significant progress in the field of antibacterial flavonoids, researchers have to turn from simply descriptive docking to the predictive, hypothesis-driven methodologies. Besides, using new and more innovative techniques like ensemble docking, explicit solvent modeling, and the testing of docking protocols with known inhibitors should become the norm in the future research. Furthermore, the integration of such studies with permeability, efflux, and target-specific biochemical assays should be a part of all related work in the future.

Machine learning-assisted docking along with QSAR modeling will be the researchers' best friends in their pursuit of prioritizing flavonoid derivatives with the right balance between potency and pharmacokinetic properties. Although it is difficult to achieve the full potential of flavonoid scaffolds, rational semi-synthetic optimization—selective prenylation, prodrug strategies, nanocarrier delivery systems, and hybrid molecule design among others—could still be a pragmatic alpha way.

Limitations of the Present Review

This review, even though it incorporates a large and modern body of literature, has some limitations never the less proprietary in vivo and clinical trial data is quite limited. However, to point out mechanistic convergence and translational relevance instead of isolated claims, this review provides a balanced and critical synthesis to direct future research on flavonoids as antibiotics.

XII. Conclusions

The review, efflux modulation, and translational potential as an interconnected approach. The However, the use of docking techniques alone usually results in the exaggeration of the biological relevance especially when the results are not confirmed through studies on permeability, efflux, and pharmacokinetics. The strongest evidence suggests that flavonoids could assist antibacterial design. Flavonoids have to be incorporated into combination therapies and successfully validated at different biological scales, otherwise they lose the battle of being a significant contributor to the global fight against antimicrobial resistance..

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